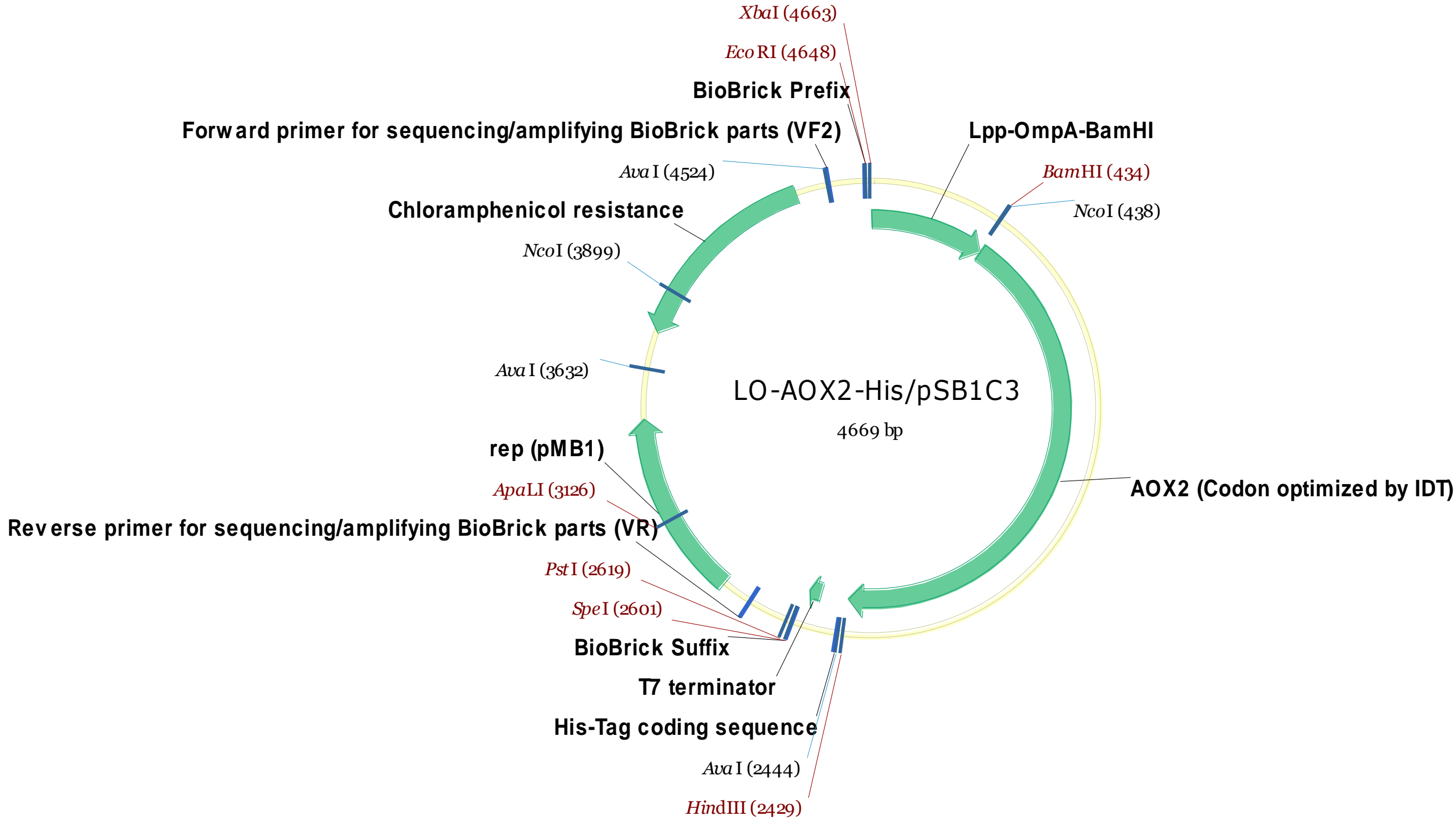


**LO-AOX2-His/pSB1C3**



# Strategy

- Vector: LO-BamHI/pSB1C3 (2509 bp) → BamHI + PstI
- Insert: AOX2-His amplified from AOX2/pET-29b (2193 bp) → BamHI + PstI

# Primer Design

- AOX2-BamHI-F:
  - 5'- AAACGAGGATCCATGGCCATCCCTGAGGAATTG -3'
- T7T-SpeI-PstI-R:
  - 5'-  
AAGAGACTGCAGCGGCCGCTACTAGTATATAGTTCCTCCTTTCAG  
-3'

# Hetero-Dimer

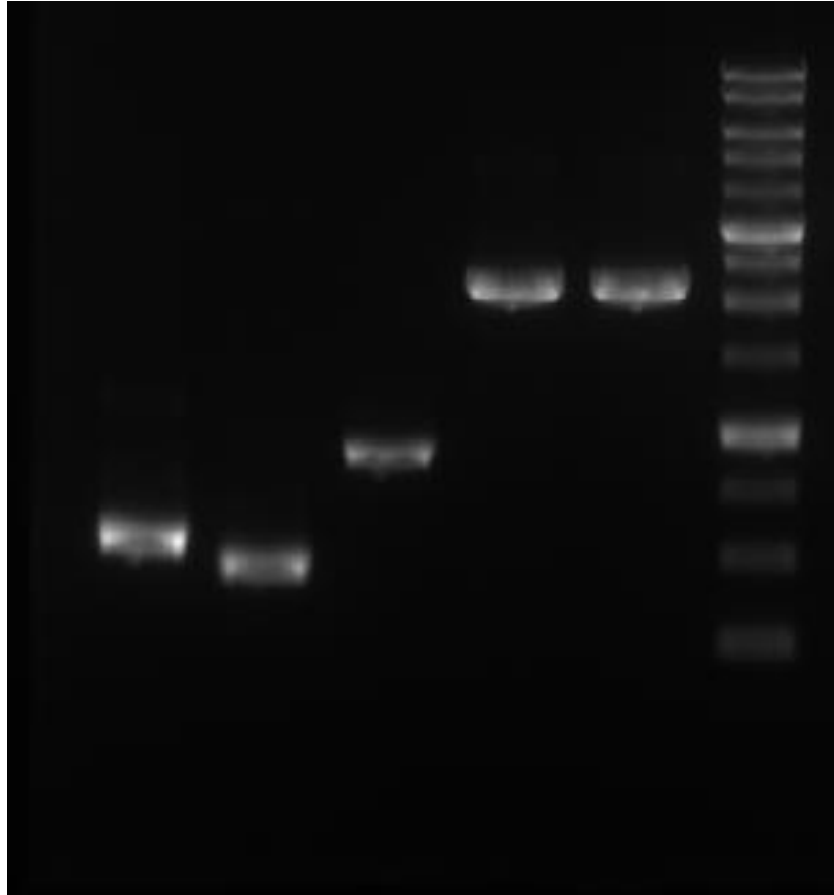
**Delta G:** -5.13 kcal/mole   **Base Pairs**4

5' AAACGAGGATCCATGGCCATCCCTGAGGAATTG

||||

3' GACTTTCCTCCTTGATATATGATCATCGCCGGCGACGTCAGAGAA

# PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

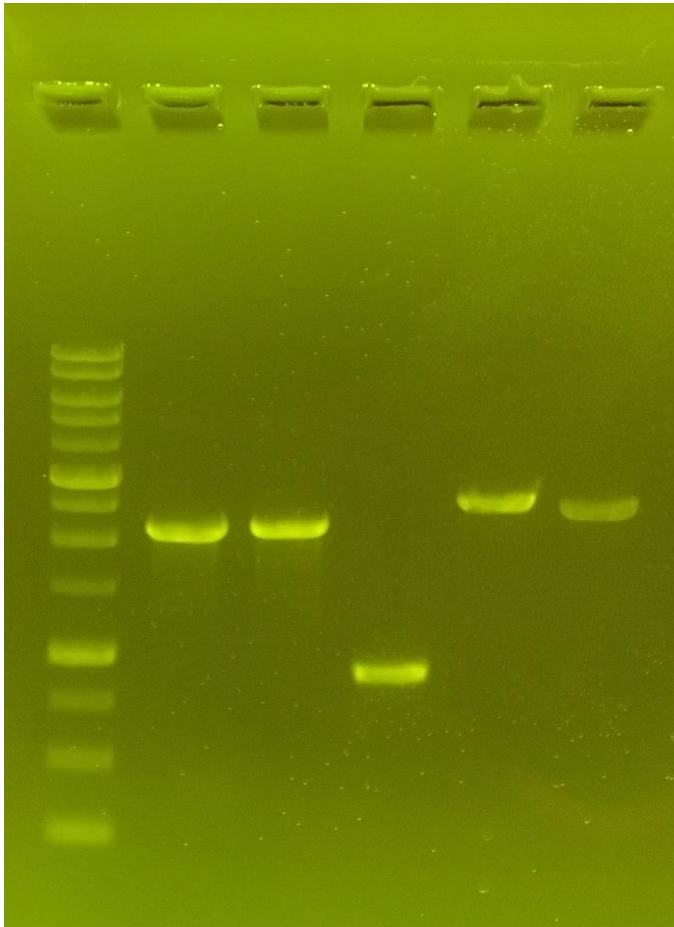
2. LO-BamHI

3. GFP

4. AOX1

5. AOX2

# Ligation



- Date: 2016.08.31

- Lane:

1. AOX1: BamHI + PstI

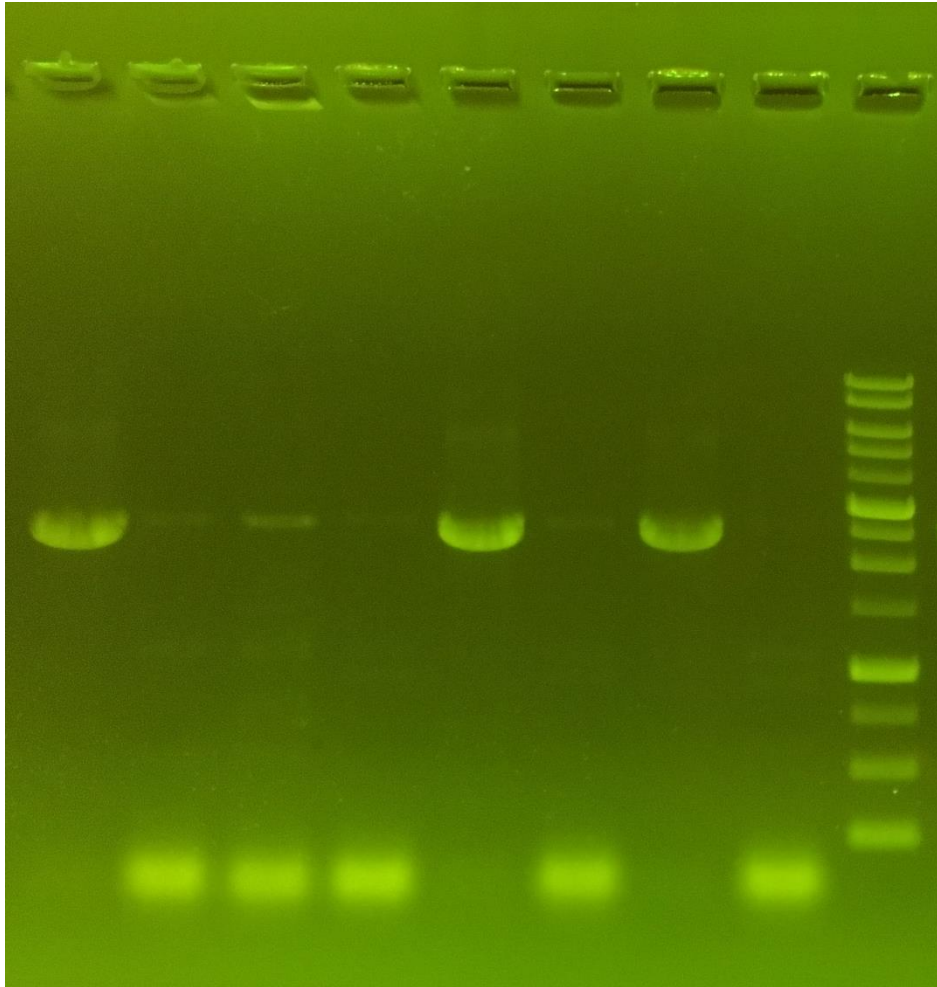
2. AOX2: BamHI + PstI

3. GFP: BamHI + PstI

4. Pcons-RBS-LO-BamHI/pSB1C3  
#P6: BamHI + PstI

5. LO-BamHI/pSB1C3 #L2: BamHI  
+ PstI

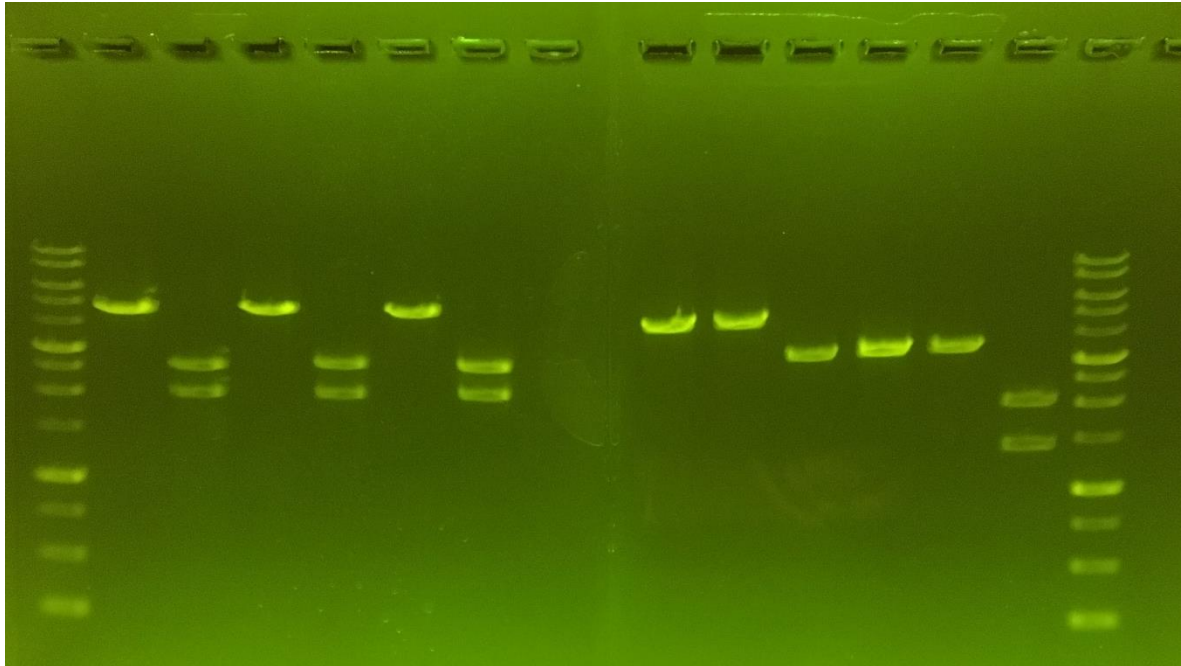
# Colony PCR



- Date: 2016.09.02
- Clone: LO-AOX2-His/pSB1C3
- Primer:
  1. Lpp-XbaI-EcoRI-F
  2. AOX2-PstI-R
- Lane: #L2-1 ~ #L2-8
- #L2-5 → Plasmid extraction → RE check



# RE check



- Date: 2016.09.07
- Clone: LO-AOX1-His/pSB1C3
- Lane:
  1. #L1-2: EcoRI
  2. #L1-2: EcoRI + PstI
  3. #P1-5: EcoRI
  4. #P1-5: EcoRI + PstI
  5. #L2-5: EcoRI
  6. #L2-5: EcoRI + PstI
  7. #P2-2: EcoRI
  8. #P2-2: EcoRI + PstI
  9. #LG-3: EcoRI
  10. #LG-3: EcoRI + PstI
  11. #PG-4: EcoRI
  12. #PG-4: EcoRI + PstI

# Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: LO-AOX1-His/pSB1C3 #L2-5
- File: “2016.09.14\_LO-AOX2-His\_pSB1C3 (#L2-5)”
- Result: correct → make a stock